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Species Variations in the Pathways of Drug Metabolism

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The basic pattern of the metabolism of foreign compounds is the same in all species, in that most foreign compounds are metabolized in two phases, in the first of which the compound may be oxidized, reduced, or hydrolyzed and in the second the products of the first phase may undergo a synthesis or conjugation to form polar excretory products. Within this pattern, however, tremendous variations can occur, since the enzymes which catalyze the reactions of these phases can be influenced by a large number of factors, species being one of the most important of these factors. Species variations in the nature and extent of the biotransformation of foreign compounds are complex and sometimes unexpected. If any foreign compound (or xenobiotic) is administered to more than one species, although one can now predict the pathways of xenobiotic metabolism, it is almost certain that species differences in the amounts of the predicted metabolites formed and excreted will be found and in some cases gross differences in the actual routes of metabolism will be found.

The metabolism of a xenobiotic is often related to its toxicity and its therapeutic activity, and since the testing of potentially useful chemicals for use in man is carried out in animals lower on the evolutionary scale than man, it is important to know whether tests on these animals can be extrapolated to man. Therefore a comparative study of metabolism in man and other species could be of value for suggesting a species likely to be useful for comparing the activity of a given compound with that in man. This is a big task, since thousands of chemical compounds are now used by man for various purposes and there are thousands of species of animals available for comparison; for example, there are 1729 species of rodents, only a few of which are extensively used for testing (e.g., the rat) and 193 species of subhuman primates which are nearest to man on the accepted evolutionary scale. Apart from testing chemicals for safe use in man, there is also the problem of the toxicity of environment chemicals to wild life. Some species may be more sensitive than others to chemical pollutants, and this difference may well be related to species differences in metabolism and how the chemical is detoxicated and excreted by the animal.

In studies, of comparative metabolism, there are obviously two main entities to be considered: the species and the compound; one can thus study the fate of one compound in many species or the fate of many compounds in one species.

The study of one compound in many species has produced certain patterns and from this type of study there has emerged the suggestion that the animals nearest to man in the pathways of drug metabolism are the subhuman primates, particularly, the Old World monkeys (*1, 2*). Examples of compounds which have been studied in this way are phenylacetic acid (*3*), sulfadimethoxine (*4*), quinic acid (*5*), coumarin (*6*), 4-hydroxy-3,5-diiodobenzoate (*7*), and methanol (*8*). These studies show that Old World monkeys and sometimes New World monkeys are similar to man in both phase I and II reactions; the 7-hydroxylation of coumarin, the oxidation of methanol, and the aromatization of quinic acid are phase I reactions, and the conjugation of phenylacetic acid with glutamine and/or glycine and of sulfadimethoxine with glucuronic acid, and the methylation of 4-hydroxy-3,5-diiodobenzoate are phase II reactions.

The study of the fate of many compounds in one species, usually the rat or rabbit, has been the principal pattern of drug metabolism research for over a century and has produced our main basic knowledge of the metabolism of foreign compounds. From this

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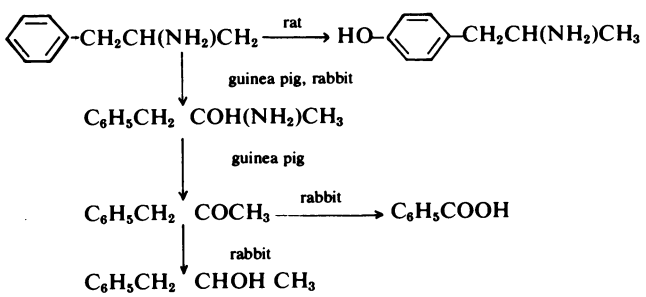
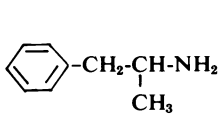
type of research has arisen the suggestion that closely related compounds should be metabolized similarly in a given species. However, this suggestion is only partly true, as illustrated below with the amphetamines, arylacetic acids, and phenols.

Comparative Metabolism of Amphetamine

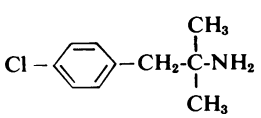
One can predict that there are two points at which amphetamine could be attacked metabolically, namely, the ring and side chain. The ring is likely to be hydroxylated mainly in the *para* position, whereas the side chain would undergo deamination and subsequent degradation. In the rat, the major metabolite of amphetamine is *p*-hydroxyamphetamine, and there is but little attack on the side chain. One could therefore predict that the main metabolic attack on other amphetamines in the rat would be *p*-hydroxylation. This has been found to be true with methamphetamine, ephedrine, norephedrine, phenmetrazine, pondinil, phentermine, and mephetermine (*9*). In the guinea pig and rabbit, there is little or no hydroxylation of amphetamine but the side chain is extensively degraded, and this is also the pattern of metabolism of other amphetamines in these species.



In chlorphentermine, the *para* position of amphetamine is blocked, and one could predict that the rat would have difficulty in metabolizing chlorphentermine, whereas the guinea pig and rabbit would attack the side chain. In fact, in the rat, chlorphentermine is slowly excreted mainly unchanged, whereas in the guinea pig and rabbit, nearly half the chlorphentermine excreted is in the form of metabolites in which the side chain NH_2 group is oxidized mainly to *N*-hydroxychlorphetermine and its *O*-glucuronide (*10*).



Amphetamine

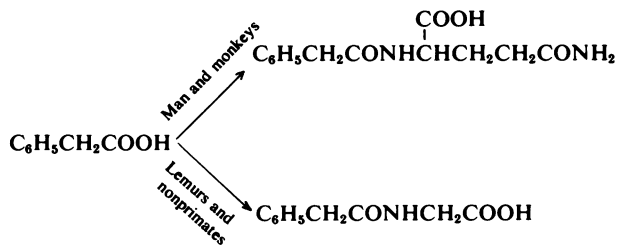


Chlorphentermine

In the metabolism of amphetamines, neither the rat nor the guinea pig is like man, but limited data on the rhesus monkey and the tamarin suggest that they metabolize amphetamine, norephedrine, and phenmetrazine more like man than any other species examined (*11*).

Comparative Metabolism of Arylacetic Acids

The basic compound here is phenylacetic acid, and it is known that this acid is metabolized by conjugation of its carboxyl group mainly with glutamine in man and monkeys (Old and New World) and with glycine in lemurs and nonprimates.



The question one can ask is, does this pattern apply to other arylacetic acids and to all primates and nonprimates? To answer such a question needs a considerable amount of work to be done with different species receiving a variety of arylacetic acids. In fact, the more species one studies, the more complicated the picture tends to become. Phenylacetic acid metabolism has been studied in some 25 species, and in most of them it is largely metabolized by conjugation, but in the Syrian hamster it is also hydroxylated in the aromatic ring, some 10% being converted to 3- or 4-hydroxyphenylacetic acid.

With five arylacetic acids, namely, phenyl-, *p*-chlorophenyl-, *p*-nitrophenyl-, indol-3-yl-, and 1-naphthylacetic acids, man and the rhesus monkey are similar, but the pattern of conjugation depends upon the arylacetic acid (see Table 1), since with phenyl-, *p*-chlorophenyl- and indolylacetic acids, the main conjugate in both species is the expected glutamine conjugate, but with 1-naphthylacetic acid the glucuronic acid conjugate is formed, and with *p*-nitrophenylacetic acid, no conjugate at all is formed in either species.

Table 2 summarizes some recent work in this laboratory on the conjugation of 1-naphthylacetic acid in 14 species. Perusal of this table suggests certain patterns. 1-Naphthylacetic acid can be conjugated at the carboxyl group with amino acids and/or glucuronic acid, the amino acid conjugations

Table 1. Conjugation of arylacetic acids in man and rhesus monkey.

Arylacetic acid	Species	% of 24 hr excretion conjugated with		
		Glutamine	Glycine	Glucuronic acid
Phenylacetic acid	Man	94	0	0
	Rhesus monkey	32	1	0
p-Chlorophenylacetic acid	Man	92	0	0
	Rhesus monkey	45	1	0
p-Nitrophenylacetic acid	Man	0	0	0
	Rhesus monkey	0	0	0
Indol-3-ylacetic acid	Man	15	0	30
	Rhesus monkey	32	0	—
1-Naphthylacetic acid	Man	0	6 (taurine)	95
	Rhesus monkey	0	2 (taurine)	89

[a]Data of Williams (*1*).

Table 2. Conjugation of 1-naphthylacetic acid in various species.[a,b]

Species	% of ^{14}C excreted conjugated with				
	Glutamine	Glycine	Taurine	Total amino acids	Glucuronic acid
Man (5 mg/kg PO)	0	0	6	6	95
Old World monkeys					
Rhesus	0	0	2	2	89
Cynomolgus	4	0	5	9	41
New World monkeys					
Squirrel	5	21	21	47	21
Capuchin	4	4	36	44	17
Marmoset	2	3	13	18	70
Lemuroids					
Bushbaby	0	53	11	64	17
Nonprimates					
Carnivores					
Dog	0	56	25	81	6
Ferret	0	7	63	70	26
Cat	0	59	40	99	0
Rodents					
Rabbit	0	6	0	6	87
Rat	0	23	1	23	51
Bats					
Indian fruit bat	0	0	0	0	94

[a]Animal dose, 100 mg/kg, IM or IP.
[b]Unpublished data of J. Caldwell, P. A. F. Dixon, and J. R. Idle.

probably taking place in the mitochondria and the glucuronic acid conjugation in the microsomes. The data in Table 2 suggest that man and the Old World monkeys conjugate it mainly with glucuronic acid, the New World monkeys (except the marmoset) and the bushbaby, mainly with amino acids and to some extent with glucuronic acid, the carnivores with amino acids extensively, the rodents mainly with glucuronic acid, and the Indian fruit bat entirely with glucuronic acid. It is to be noted also that the actual amino acid used varies between glycine and taurine. Taurine conjugation is predominant in carnivores and New World monkeys and glycine conjugation also in the carnivores (dog and cat) and the bushbaby. Table 2 shows that there are vague patterns, but at the same time each species must be treated separately. This is illustrated by the ferret having the most extensive taurine conjugation and

the marmoset being more like man and the Old World monkeys than the New World monkeys to which it belongs.

Comparative Metabolism of Phenols

It is well known that simple phenols are metabolized and detoxicated by conjugation with sulfate and glucuronic acid, but some common species are known to have defects in these conjugations. The domestic cat, for example, has a defective glucuronic acid conjugation (*12*) which may also occur in other members of the cat family such as the lion, civet, and genet (*13*). In fact, the cat seems to be more sensitive to phenol than other common laboratory animals. The cat metabolizes phenol by conjugation with sulfate. The pig, on the other hand, seems to have a defective sulfate conjugation of phenol and metabolizes phenol by conjugation with glucuronic acid. These defects, however, are not universal for all phenols, since the cat conjugates phenolphthalein more with glucuronic acid than sulfate and the pig shows a substantial sulfate conjugation of 1-naphthol as shown in Table 3. The rat uses both conjugations almost equally well with the phenols. It is to be noted that the pig seems to distinguish between 1- and 2- naphthol, in that 2-naphthol is almost entirely excreted as the glucuronide whereas about a third of 1-naphthol is eliminated as a sulfate. This type of observation suggests that, in species variation studies, it is important to define specifically the substrate as well as the species. Thus in the case of the cat, it is not entirely true that it has a defective glucuronic acid conjugation for all compounds that can form glucuronides. A small number of compounds are now known to be extensively conjugated with glucuronic acid in the cat, e.g., diphenylacetic acid which is conjugated to the extent of 70% of the dose with glucuronic acid and hydratropic acid where the conjugation is 40% (P. Dixon, J. Caldwell, R. L. Smith, unpublished data).

An interesting observation on the conjugation of 1-naphthol in monkeys has recently been made in this laboratory. In two species of Old World monkeys, the rhesus monkey and cynomolgus monkey, [1-^{14}C]-1-naphthol (10 mg/kg) was found to be excreted mainly as the sulfate conjugate, whereas in two species of New World monkeys, the capuchin and tamarin, the same dose was excreted mainly as the glucuronide (see Table 4) (P. C. Hirom and R. Mehta, unpublished data). This supports earlier work on phenol (*14*) which was shown to conjugate mainly with sulfate in the rhesus monkey and with glucuronic acid in the capuchin and squirrel monkeys.

Table 4. Conjugation of [1-^{14}C]-1-naphthol in monkeys.[a,b]

Monkey (sex)	% dose excreted in 24 hr	% of 24 hr excretion of ^{14}C	
		As sulfate	As glucuronide
Old World			
Rhesus (1M, 1F)	99, 101	83, 86	14, 12
Cynomolgus (1F)	97	76	23
New World			
Tamarin (1M)	84	15	84
Capuchin (2F)	26, 32	2, 0	97, 99

[a]Data of P. C. Hirom and R. Mehta (unpublished).
[b]Dose: 10 mg/kg, intramuscularly.

Dose and Pattern of Drug Metabolism

The size of the dose of a xenobiotic could alter the pattern of its metabolism in so far as a large dose could exhaust the mechanism by which a small dose is metabolized. The compound could then be partly

Table 3. Conjugation of phenols with glucuronic acid (GA) and sulfate (S) in the rat, cat, and pig.[a]

Labeled phenol	% of 24 hr excretion of labeled phenol					
	Rat		Cat		Pig	
	Conjugated with GA	Conjugated with S	Conjugated with GA	Conjugated with S	Conjugated with GA	Conjugated with S
Phenol	44	55	1	95	94	6
1-Naphthol	47	53	1	98	64	33
2-Naphthol	52	48	3	97	94	6
Phenacetin[b]	22	71	3	86	54	10
Phenolphthalein			60	40		

[a]Data of Williams (*1*).
[b]Phenacetin is excreted mainly as 4-acetamidophenol (acetaminophen).

excreted unchanged or undergo another metabolic reaction. Changes in metabolic reactions are quite common with phase II or conjugation reactions, especially where certain conjugating agents are limited in supply, e.g., glycine and sulfate.

For example, it has been known for a considerable time that in man and the pig, small doses of benzoic acid are metabolized entirely by conjugation with glycine, but with large doses, glucuronic acid conjugation become predominant (*15*). The cat, as already mentioned, has a defective glucuronic acid conjugation for certain compounds including benzoic acid. Consequently, benzoic acid is more toxic to the cat than most common species of animals (*16, 17*), for the cat conjugates benzoic acid entirely with glycine, which is limited in supply, and glucuronic acid conjugation is not available to take over when large doses of benzoic acid are administered. The effect of dose on the glycine conjugation of phenylacetic acid in the ferret (J. R. Idle, unpublished data) and of 1-naphthylacetic acid in the rat (J. Caldwell and P. Dixon unpublished data) have been studied recently in this laboratory in connection with taurine conjugation which is a relatively new mechanism. Table 5 shows the pattern of conjugation of phenylacetic acid in the ferret with varying doses, and suggests that glycine conjugation is used mainly at the lowest dose, then as the dose increases taurine conjugation becomes evident and at higher doses still glucuronic acid conjugation is also taking place. This suggests that glycine tends to be exhausted first and then taurine and finally glucuronic acid is utilized.

Table 5. Effect of dose on the conjugation of phenylacetic acid in the ferret.[a]

Dose, mg/kg	% of 24 hr excretion conjugated with		
	Glycine	Taurine	Glucuronic acid
0.01	69	8	0
100	63	21	0
200	41	38	4
400	36	33	10

[a]Data of Idle (unpublished).

Table 6 shows the interesting case of the effect of dose on the pattern of conjugates in the urine and the bile of bile duct-cannulated rats receiving 1-naphthylacetic acid. It is clear from the figures for urine that glucuronic acid conjugation is taking over from glycine conjugation as the dose rises, but for the bile the pattern is practically unchanged for all the doses. The bile pattern is explained by the fact that 1-naphthylacetylglucuronide (molecular weight 362) is extensively excreted in the bile in the rat, whereas 1-naphthylacetylglycine (molecular weight 231) is not. The molecular weight of the glucuronide is within the range 325 ± 50 given by Hirom et al. (*18*) for extensive biliary excretion in the rat, whereas that of the glycine conjugate is below this range, and this conjugate is unlikely to be excreted in the bile in large quantities.

Table 6. Effect of dose on the urinary and biliary excretion of conjugates of 1-naphthylacetic acid.[a,b]

Dose, mg/kg	% of 3 hr excretion			
	In urine		In bile	
	Glycine conjugate	Glucuronic acid conjugate	Glycine conjugate	Glucuronic acid conjugate
5	87	11	4	90
25	55	44	2	91
50	48	52	1	94
250	12	85	1	92
500	11	88	1	85

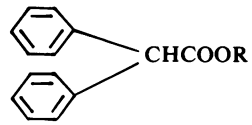
[a]Data of P. A. F. Dixon and J. Caldwell (unpublished).
[b]Bile duct-cannulated rats injected intraperitoneally. Molecular weights: 1-naphthylacetylglucuronide = 362; 1-naphthylacetylglycine = 231.

Many other examples of changes in conjugation with dose are to be found in the literature. Perhaps a most interesting one is that of *p*-acetamidophenol (acetaminophen, panadol, paracetamol), which produces liver necrosis in high doses. This drug forms a sulfate, a glucuronide, and a mercapturic acid. The toxic effect of high doses appears to be related to the depletion of the liver of glutathione which is used to form the mercapturic acid. Studies in the hamster, a species most sensitive to the necrotic effects of *p*-acetamidophenol, have shown that at low doses more of the drug is excreted as sulfur conjugates—sulfate and mercapturic acid—than glucuronide, but at high doses the reverse is true (*19*).

It is clear from the study of the effect of dose on glycine, sulfate, and glutathione conjugations that these mechanisms have a limited capacity unless supplemented from outside sources and that when they are exhausted the glucuronic acid mechanism takes over. It follows from this that, with any compound which is detoxicated by conjugation with glucuronic acid only, the pattern of metabolites will not change very much with dose unless this is excessive. This has been found to be the case with diphenylacetic acid and hydratropic acid (2-methyl-2-phenylacetic acid) in the rat.

These acids are conjugated extensively with glucuronic acid and at both low and high doses.

The metabolism of amphetamine in humans of similar weight is much the same at 20 mg as at 100 mg per subject (*20*).



Diphenylacetic acid (R=H)
Diphenylacetylglucuronide ($R=C_6H_9O_6$)

Hydradratropic acid (R=H)
Hydratropoylglucuronide ($R=C_6H_9O_6$)

In general, it seems probable that the extent of metabolic pathways will change with increase in dose, since it is to be expected that different mechanisms have different capacities, and furthermore, some compounds can induce their own metabolism and possibly one pathway more than another. These changes, however, may also vary with species as in the case of the cat with its defective glucuronic acid mechanism. Some effects of high doses and continuous dosing of certain foreign compounds are shown in Table 7.

Table 7. Toxic effect from high or continuous doses.

Compound	Species	Toxicity	Probable cause
p-Acetamidophenol	Several	Liver necrosis	Glutathione depletion
Bromobenzene	Several	Liver necrosis	
Benzyl chloride	Rat	Growth inhibition	
Naphthalene	Rabbit	Cataract	
Benzoic acid	Cat	Poisoned	Glycine depletion; lack of glucuronyl transferase
Phenol	Cat	Poisoned	Sulfate depletion; lack of glucuronyl transferase
Nicotinamide	Rat	Growth inhibition	"Methyl deficiency"

The body can probably effectively detoxicate "small and moderate doses" of the majority of foreign compounds, although the magnitude of "small and moderate doses" will vary with the compound and the species. However, more work is necessary to find out how many times a dose must be increased before a change in metabolic pathway occurs. Is the factor 2, 5, 10, 100, or more?

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